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EFFECT OF SODIUM CHLORIDE ON STEVIA PLANT GROWTH IN VITRO

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Abstract

The research was conducted to study the effect of sodium chloride on the growth of stevia plants in vitro, the experiments included the selection of two types of plant parts, the middle cutting and the shoot tips, and the effect of different concentrations of sodium chloride on the number of nodes and branches, the fresh weight of the plant and the height of the plant. The plantings were established on MS medium, and the results showed that the effect of the plant parts was significant if the media cutting gave the highest average number of nodes of 6.2 nodes per plant. As for the interaction between the plant part and the concentration of sodium chloride, the measurement treatment was superior when using the middle cutting, as it recorded the highest rate of nodes of 10 nodes per plant and the highest rate of number of branches reached 2.6 branches/plant, and the highest rate of fresh weight of the plant amounted to 1.106 gm/plant compared to the measurement treatment when using shoot tips , which recorded the lowest rate for each of the above traits, a significant decrease in the number of nodes and branches, plant height and plant fresh weight was noted with an increase in the concentration of sodium chloride.

Keywords: In Vitro, Stevia Plant and Sodium Chloride.

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Introduction

Environmental conditions are one of the main factors affecting crop production and food security. The problem of salinity is one of the most prevalent harmful environmental problems that limit crop productivity as it severely hampers plant growth and development through changes in morphological, biochemical and molecular features. More than half of agricultural land is located In arid and semi-arid regions, many researches have been conducted to study the harmful effects of salinity on the stevia plant. In these studies, it was found that stevia plants were affected by different levels of salinity and that this plant is relatively sensitive to salt stress (kurunc et al, 2020).

Stevia is a herbaceous plant that is classified as a perennial plant in tropical or subtropical regions, where it stays in the ground for up to seven years, but in cold regions it is considered an annual crop harvested once before winter. Cantabella et al (2017) The stevia plant belongs to Composite ،The stevia plant is one of the plants that has a super sweetening power that reaches more than 300 times the sweetening of ordinary sugar used in food, it does not contain any calories in its leaves, and therefore it is ideal for sweetening foods used by patients, and there is stevioside in its leaves which is responsible for the sweet taste) Mizutani, Tanaka 2002).

Gupta et al. (2014) study The effect of salt stress on stevia plant lime. The results of the study indicated the moral effect, as the highest rate of stephol glycoside was 1.57 and 1.43% on relay when adding 0.10% and 0.025% of each of sodium chloride and Na2Co3 , managed Abdul Razak et al. (2014) from obtaining the highest rate of the number of branches reached 7.8 branch/vegetable part , When adding 0.5 mg/L KIN and 0.25 mg/L BAP , In a study conducted by Mahyar et al. 2020,in using the effect of chitosan elicitor at four different concentrations (0, 0.2, 0.4 and 0.6 gm/L) on the physiological and biochemical properties of stevia plant under four levels of salinity stress (0, 50, 100, 150). millimoles of sodium chloride). It was observed that salt stress reduced chlorophyll a (chlorophyll a), chlorophyll b (chlorophyll b), total chlorophyll, carotenoid and total protein content. In the field of stevia propagation in vitro, the effect of salinity and drought stress on growth, physiological response and biochemical content was studied. The cultures were created on MS medium supplemented with different concentrations of 6-benzylaminopurine (BAP) and kinetin (Kn) individually and/or in combination, when exposing plants to NaCl and mannitol, a significant decrease was observed in the shoot number, root length, root number and root length with an increase in the concentration of NaCl and mannitol (25, 50, 75, 100 mmol), while a significant increase was observed in all measurements with the increase in the number of days. (10, 20, 30) compared to the measurement treatment (Mukeshwar and Chikara, 2014). Therefore, the aim of the research is to study the effect of salt stress on the growth of the stevia plant in vitro.

Materials and methods

Preparation of the plant parts: Vegetative branches with a length of 15-20 cm were taken from stevia seedlings, the leaves and roots were removed from them, the ends of the branches and stem nodes of 0.5 cm length were separated and cleaned with running water and liquid soap and sterilized with a solution of sodium hypochlorite NaOCl at a concentration of 3% for 15 minutes with the addition of drops of 20 Tween was then washed with sterile distilled water three times. The excised plants were washed in sterile double-distilled water and grown on a multivitamin supplement, 3% sucrose(Hilo et al, 2020).

Initiation stage

The plant parts were grown on MS medium (Murashige and Skoog, 1962) equipped with NAA and kin at a concentration of 0.3 mg/L and the plants were incubated at 25 ◦C under 1000 lux for a light period of 16/8 light/dark for four weeks.

Shoot multiplication stage

The resulting branches were transferred from the germination stage to MS medium prepared with / kin at a concentration of 0.5 mg/L with 0.2 mg/L of NAA, and NaCl was added to the medium at different concentrations (0, 50, 100 and 150 mg/L).

The cultures were incubated at a temperature of 25°C under 1000 lux and for a period of 16/8 light/darkness for a period of four weeks, after which the measurements were taken.

Characters studied

1- The average number of nodes formed

2- The average number of branches

3- The average length of the branch

4- Average weight of the soft plant

Experimental design and statistical analysis

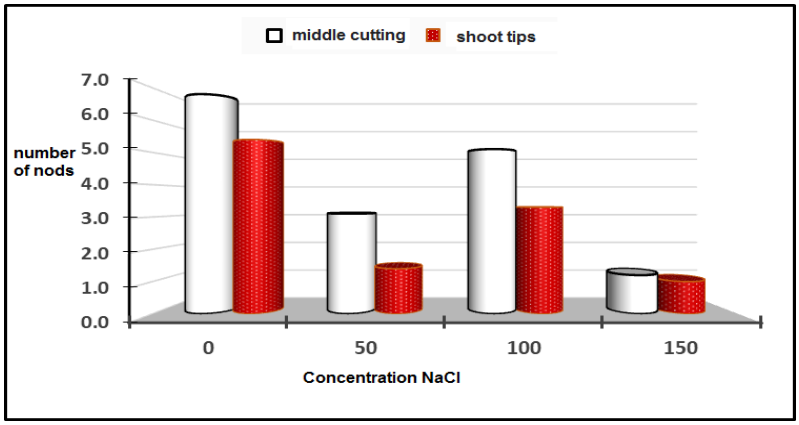
The data of the experiments were analyzed as factorial experiments and with ten replications for one treatment using the Complete Randomized Design (CRD) and the averages were compared according to the least significant difference test at the probability level of 0.05 (Al-Sahoki and Waheeb, 1990).

Results and discussion

number of nodes

The results of Figure (1) show that the inclusion of the nutrient medium with sodium chloride salt at a concentration of 150 mg/L was significant in the decrease in the number of knots, as the lowest rate was recorded at 1.1 knots/plant compared to the measurement treatment that recorded the highest rate at 7.9 knots/plant. As for the effect of the part. The average nodes outperformed the average number of knots at 6.2 knots/plant compared to the growing top, which recorded the lowest rate of 2.9 knots/plant. The interaction treatment between the growing top and sodium chloride at a concentration of 50 and 150 mg/l gave the lowest rate of cuttings at 1.0 knots/l. plant compared to the middle cutting treatment at a concentration of 0.0 mg/liter of sodium chloride, which recorded the highest rate of 10.0 knots/plant. Salt stress leads to decreased growth through an inhibitory effect on food transformation activities, osmotic imbalance, apparent deficiency in the absorption of nutrients, decreased protein synthesis and the destruction of developing plant cells, and thus the cells do not perform their usual function. This result is consistent with what was found by Kamel et al.,(2016).

Figure (1): Effect of Nacl concentrations and explant and their interaction on the average number of nods formed of stevia branch multiplication after four weeks of culture



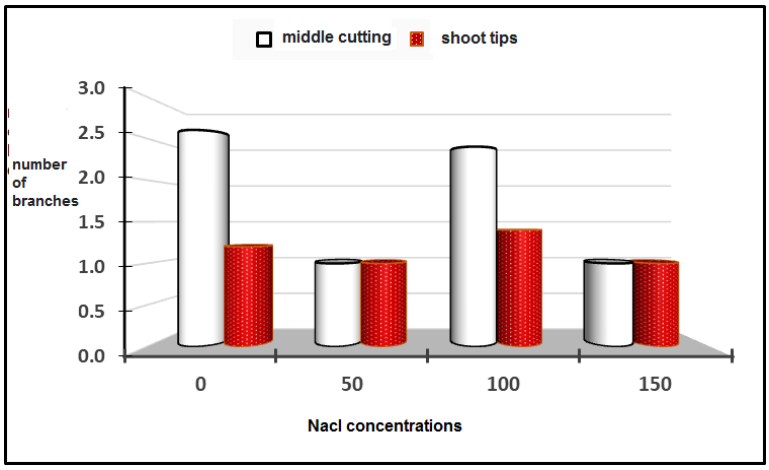
Number of Forks

The results of Figure (2) indicate that the highest rate of branching was 1.9 branches/plant at the concentration of 100 mg/L of sodium chloride and when the control treatment compared with the lowest rate of the number of branches was 1.0 branches/plant in the treatments of 50 and 150 mg/L of chloride Sodium, as for the effect of the part. The average cutting outperformed in the average number of branches reached 1.8 branches/plant compared to the growing top, which recorded the lowest rate of 1.2 branches/plant

As for the interaction effect between the plant part and sodium chloride, the treatment of the median cutting at the concentration of 0.0 mg / liter of sodium chloride recorded the highest rate of 2.5 branch / plant

Salinity has a negative effect on the accumulation of cytokinins and gibberellins in conjunction with the activation of natural growth inhibitors such as abscisic acid, which is negatively reflected on plant growth represented by a clear decrease in leaf area and consequently a decrease in the efficiency of the photosynthesis process as well as a clear decrease in the hormonal content. (Abu Zaid, 2000), and the increase in salinity leads to a decrease in the rate of cell division and elongation that is controlled by auxins that are finally inhibited by the effect of salt stress (Munns and Tester, 2008).

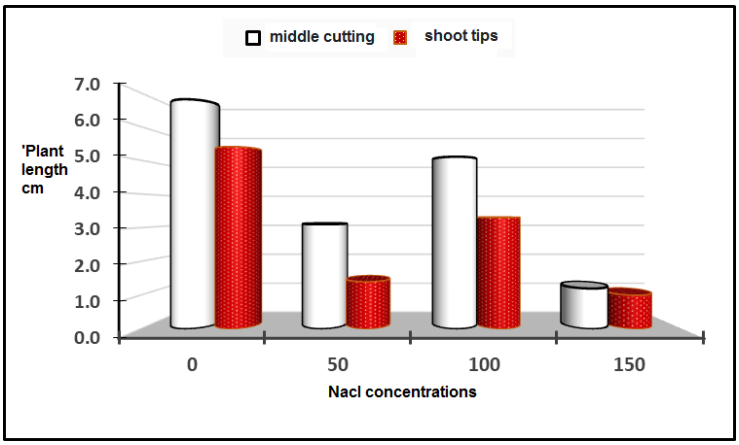
Figure (2): Effect of Nacl concentrations and explant and their interaction on the average number of fork formed of stevia branch multiplication after four weeks of culture



Plant length

The results of Figure (3) showed the moral effect of the plant part on increasing the length of the branch, and the treatment of the middle cutting was superior by giving the highest rate of branch length of 4.1 cm compared to the growing top, which gave the lowest rate of length of 2.8 cm, as for the concentration of sodium chloride, it gave the treatment with a concentration of 150 mg/L The least average branch length was 1.1 cm compared to the measurement treatment, which gave the highest rate of branch length was 6.1 cm, and the interaction between the concentration of sodium chloride and the plant part was the treatment of the growing top and the concentration 150 mg/L gave the lowest rate of branch length was 1.0 cm compared to the measurement and middle cutting treatment Which gave the highest rate of 6.8 cm

Figure (3): Effect of Nacl concentrations and explant and their interaction on the average of 'Plant length' formed of stevia branch multiplication after four weeks of culture



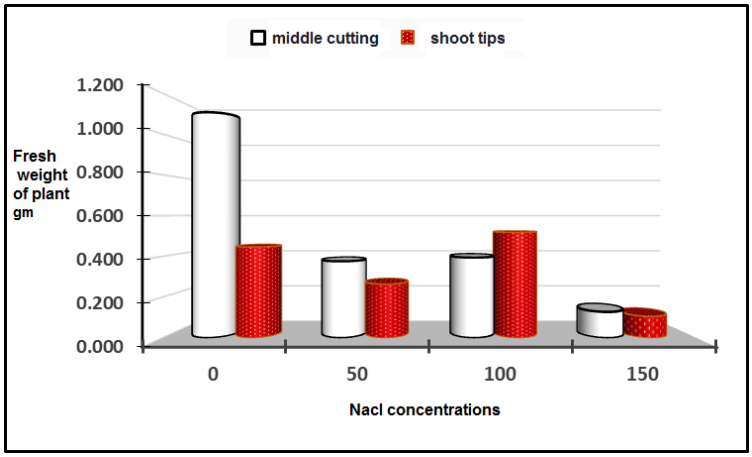
Fresh weight

Figure (4) shows the effect of the plant part on the weight of the fresh plant, as the treatment of the middle cutting was superior in giving the highest rate of the fresh weight of 0.501 gm, compared to the treatment of the growing top, which gave the lowest rate of plant weight of 0.334 gm. As for the concentration of sodium chloride, the concentration treatment

was recorded as 150 mg / liter of sodium chloride, the lowest rate of plant weight was 0.117 gm, compared to the measurement treatment, which recorded the highest rate of plant weight, which was 0.776 gm. As for the interaction treatments, the measurement treatment and the middle cutting, which gave the highest rate of plant weight, reached 1.106 g, compared to the interaction treatment between the top growing The concentration was 150 mg/L of sodium chloride, which gave the lowest rate of 0.106 gm.

Studies on salt stress indicate that high salinity in the growing medium causes a significant decrease in leaf area, length, dry and wet weight of the vegetative and root groups, and decreases the content of chlorophyll and carotenoids, and then decreases the activity of photosynthesis. The reason for the decrease in the fresh weight of plants in high concentrations of NaCl may be due to A decrease in the water effort in the growth medium of the root system, which reduced the ability of the plant to absorb water as well as accompanied by a decrease in the absorption of nutrients from plant cells and led to a decrease in the wet weight of the plant (Evers et al., 1998).

Figure (4): Effect of Nacl concentrations and explant and their interaction on the average of Fresh weight of the plant formed of stevia branch multiplication after four weeks of culture



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